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RAPID COMMUNICATION

Expression of T Cell Receptor Delta Chains in Benign and Malignant T Lineage Lymphoproliferations

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Recent studies in both human and murine systems have demonstrated the existence of a second CD3-associated T cell receptor (the γ δ-TCR) distinct from the αβ heterodimer associated with antigen recognition by classical T cells. Using a monoclonal antibody specific for the δ component of the human γδ-TCR, the expression of this antigen in both benign, reactive lymphoid tissues and T lineage lymphomas was studied with immunohistologic techniques. In the normal thymus, TCR-δ^+ cells constituted less than 5% of the CD3$^+$ thymocytes and were located primarily in the medulla or juxtamedullary cortex. Within the T zones of 16 histologically varied reactive peripheral lymphoid tissues, including four patients with marked predominantly paracortical hyperplasia, the authors identified from less than 1% to a maximum of 5% TCR-δ^+ cells. While these results are consistent with the hypothesis that TCR-$\gamma\delta^+$ cells comprise a small distinct subpopulation of peripheral T cells in humans, selective localization or recruitment of these cells could not be demonstrated in any of a number of tissues or reactive situations. Among 62 T lineage lymphomas, including 14 CD3$^+$/TCR-β^- cases, only two TCR-δ^+ neoplasms were identified, both lymphoblastic lymphomas displaying the CD3$^+$/CD4$^-$/CD8$^-$ phenotype known to be associated with normal TCR-$\gamma\delta^+$ T cells. Because the majority of CD3$^+$/TCR-β^- lymphomas did not display TCR-δ, these results argue against the hypothesis that the high incidence of CD3/TCR-β discordance noted in T lineage lymphomas represents preferential transformation of the TCR-δ-expressing subset. (Am J Pathol 1988, 132:401–405)

ANTIGEN-SPECIFIC, MAJOR histocompatibility complex-restricted recognition by classical T cells has been shown to be mediated by a clonotypic αβ heterodimer noncovalently associated with the nonpolymorphic CD3 complex.[1] Although this αβ T cell receptor (TCR) is expressed on the surface of the great majority of CD3$^+$ peripheral T cells,[2–4] recent studies indicate that approximately 2–4% lack αβ dimers.[4,5] Furthermore, about a third of a large series of human T lymphomas lacked expression of TCR β-chains.[3]

Recent evidence at both the DNA and protein level has suggested that normal TCR-$\alpha\beta^-$ cells bear a second distinct, CD3 associated, functionally competent TCR.[6,7] This second TCR has been named the γδ heterodimer, and monoclonal antibodies specific for both the human γ and δ chains have been produced.[8,9] One of these antibodies, specific for the human TCR-δ chain,[9] works well in tissue section immunohistology, and in this study this reagent was used to analyze expression of TCR-δ chains in a large series of benign and malignant T cell proliferations. These results con-

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Table 1—T Lineage Lymphomas: Histologic and Immunophenotypic Features

Histology	No. of cases		Immunophenotypes: "Pan" T antigen expression (No. of cases) CD2	CD3	CD5	CD7	TCR-B	CD4/CD8 antigen expression (No. of cases)* +/−	−/+	+/+	−/−
Peripheral T lymphomas (PTL)											
Immunoblastic	9	PTL (41)	35	34	22	12	29	28	5	1	5
Large cell	5	TTL (21)	17	20	21	19	13	2	0	13	6
Mixed	7										
AILD-like	8										
Monomorphic medium-sized	4										
Mycosis fungoides	8										
	41										
Thymic T lymphomas (TTL)											
Lymphoblastic	21										
TOTAL	62										

* Two cases of PTL not interpretable for CD4/CD8 expression.

firm the existence of a small population of TCR-δ^+ cells in the T zones of normal and reactive lymphoid tissues; however, expression of TCR-δ did not account for the high incidence of the CD3$^+$/TCR-β^- phenotype in T lineage lymphomas.

Materials and Methods

Sixty-two cases of T lineage lymphoma, 16 lymphoid hyperplasia, and a variety of normal tissues were examined in this study. Cases were diagnosed and classified using both histologic and immunologic criteria, as described previously.[10,11] The histologic and immunologic features of the T lineage lymphomas studied are presented in Table 1. The reactive cases examined included 12 cases of predominantly follicular hyperplasia (6 lymph node, 2 tonsil, 1 spleen, 1 skin, 1 nasopharynx, and 1 lung), and 4 cases of predominantly paracortical hyperplasia (all lymph node). The normal tissues studied included thymus, spleen, ileum, colon, appendix, kidney, lung, brain, liver, and skin.

Specimens were processed and immunostained using a 3-stage biotin-avidin-peroxidase procedure, as described previously.[11] Each case (excluding normal nonlymphoid tissues) was studied with a large panel of monoclonal antibodies against T lineage differentiation antigens as well as antibodies reactive with B lineage cells, macrophage/dendritic reticulum cells, and proliferating cells (see Picker et al[3,11] for specific reagents). This analysis was necessary for classification purposes, as well as for delineating the neoplastic population within the cryostat sections. CD3 (Leu 4),[11] anti-TCR-β (βF1),[2] and anti-TCR-δ-1 were studied on serial sections. The production and characterization of the TCR-δ-1 specific monoclonal antibody has been described.[9] This antibody reacts with either a framework determinant of TCR-δ chains or a very common variable region determinant: all known $\gamma\delta$ cell lines ($\sim$100), including those previously thought to have CD3 associated $\gamma\gamma$ homodimers, are positive for anti-TCR-δ-1 (unpublished data). For TCR-δ to be called positive in a T lymphoma, at least 10% of the morphologically or immunologically-defined neoplastic population was stained with the anti-TCR-δ-1 monoclonal antibody.

Results

As reported previously,[3] the vast majority of non-neoplastic CD3$^+$ T cells display TCR-β chains. Tissue section immunohistology of 16 reactive lymphoproliferations, as well as normal spleen, gut-associated lymphoid tissue, and thymus, revealed nearly identical staining patterns for CD3 and TCR-β. In contrast, TCR-δ^+ cells varied from 1% or less of CD3$^+$ cells to a maximum average frequency of about 5%. In peripheral lymphoid tissues, the distribution of TCR-δ^+ cells paralleled that of the predominant TCR-β^+ T cells. The great majority of TCR-δ bearing cells were in the T zones of these lymphoid tissues (lymph node paracortex, splenic periarteriolar lymphatic sheaths, etc.) with only occasional TCR-δ^+ cells in primary or secondary B cell follicles (Figure 1A). In the thymus (Figure 1B), the frequency of TCR-δ^+ cells was greatest in the medulla (approximately 3–5% positive). Within the cortex (approximately 1% positive overall), TCR-δ^+ cells were usually situated near the cortical–medullary junction, rather than in the subcapsular zone. None of the nonlymphoid tissues examined showed staining with TCR-δ. The intraepidermal

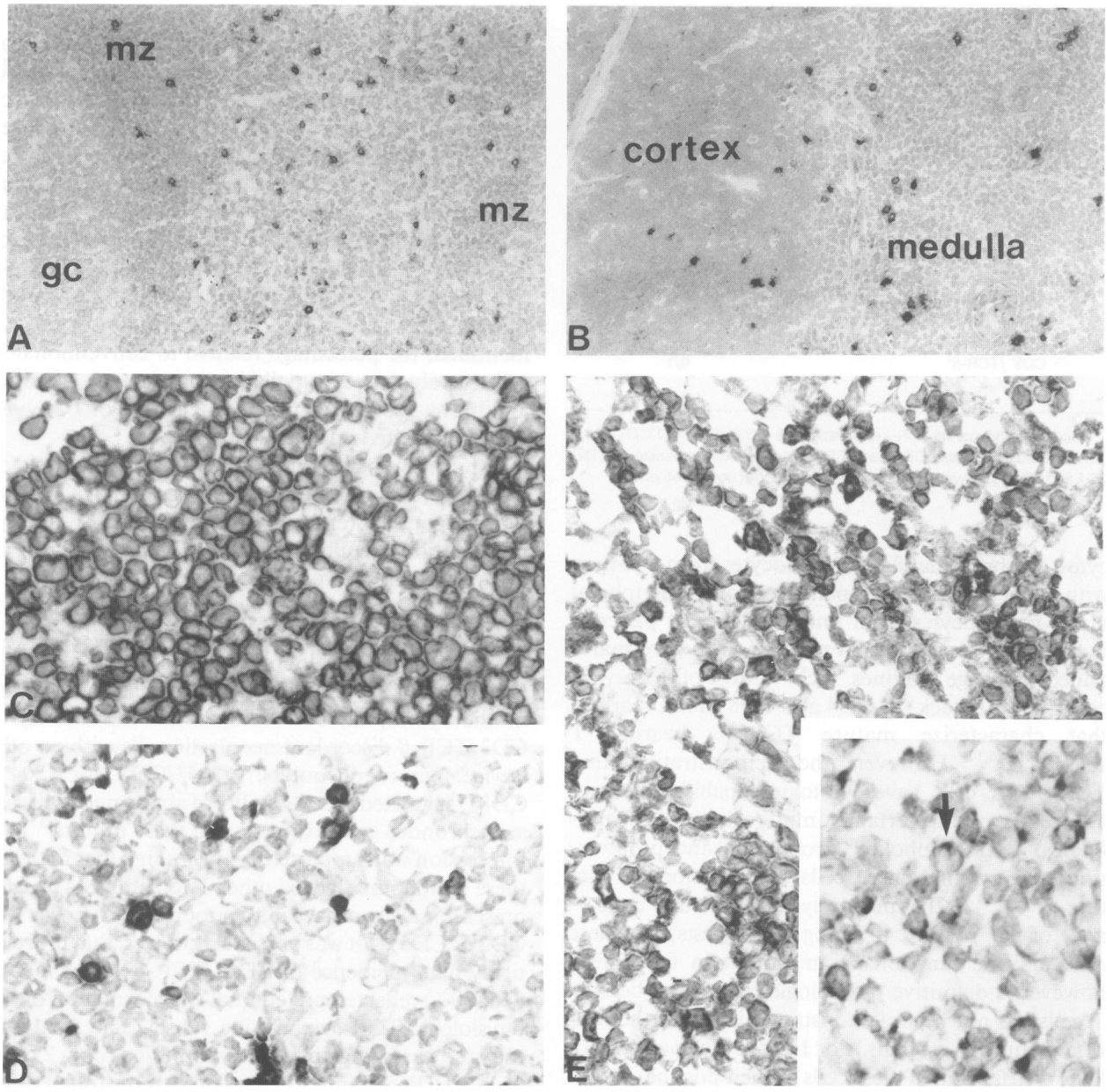

Figure 1—A—Distribution of TCR-δ^+ cells in reactive tonsil. The number of positive cells represents the maximum observed in reactive peripheral lymphoid tissues. gc, germinal center; mz, mantle zone. **B**—Distribution of TCR-δ^+ cells in normal thymus. The number of TCR-δ^+ cells is greatest in the medulla and juxtamedullary cortex. **C–E**—T lymphoblastic lymphoma stained with CD3 (C), TCR-β (D), and TCR-δ (E). Neoplastic cells show CD3 and TCR-δ expression, but lack TCR-β. The scattered TCR-β^+ cells represent reactive (normal phenotype) cells within the neoplasm. (Inset) The second example of a TCR-δ^+ lymphoma showed focal, dotlike cytoplasmic staining (arrow) in a subset of neoplastic cells.

Thy-1^+, TCR-$\gamma\delta^+$ dendritic cell population reported in the mouse[12] did not appear to have a human counterpart, as anti-TCR-δ-1 did not stain normal human epidermis at all.

The 62 T lineage lymphomas examined included a variety of histologic and immunologic types (Table 1). None of the 41 peripheral T cell lymphomas studied, including 7 cases with a $CD3^+/TCR\text{-}\beta^-$ phenotype, expressed TCR-δ (Table 2). Of 21 "thymic" (T lymphoblastic) lymphomas, two showed TCR-δ expression, one with the majority of neoplastic cells positive (Figures 1C–E), and the other with partial expression (25–30% positive). The pattern of TCR-δ^+ expression in the latter case was unusual; most staining appeared cytoplasmic with many cells showing focal, intense, dotlike positivity (Figure 1E, inset). Both TCR-δ^+ T lymphoblastic lymphomas were negative for the CD4 and CD8 antigens.

Discussion

In humans, CD3-associated γδ-TCR have been demonstrated on normal peripheral blood lympho-

Table 2—Expression of T Cell Receptor Delta Chains in T Lineage Lymphomas

	TCR-δ^+/Total
Peripheral T lineage lymphomas	
$CD3^+/TCR\text{-}\beta^+$	0/27
$CD3^+/TCR\text{-}\beta^-$	0/7
$CD3^-/TCR\text{-}\beta^+$	0/2
$CD3^-/TCR\text{-}\beta^-$	0/5
TOTAL	0/41
Thymic T lineage lymphomas	
$CD3^+/TCR\text{-}\beta^+$	0/13
$CD3^+/TCR\text{-}\beta^-$	2/7*
$CD3^-/TCR\text{-}\beta^+$	-
$CD3^-/TCR\text{-}\beta^-$	0/1
TOTAL	2/21

* One case with partial TCR-δ expression (25–30%). Both $CD3^+/TCR\text{-}\beta^-$/TCR-δ^+ cases were also $CD5^+$ and $CD9^+$, but lacked expression of CD2, CD4 and CD8. CD1 was expressed in one case, but not the other.

cytes, cerebrospinal fluid lymphocytes from a patient with subacute sclerosing panencephalitis, peripheral blood lymphocytes from immunodeficient patients, normal thymocytes, and a number of *in vitro* maintained T lineage cell lines.[4,8,9,13–17] Most of these $\gamma\delta$ T cells appear to lack the CD4 and CD8 subset markers that characterize mature TCR-$\alpha\beta$ bearing T cells.[5,13,14,16,17] However, the demonstration that TCR-$\gamma\delta^+$ T cells have cytotoxic capability and are able to respond to certain stimuli (eg, anti-CD3 antibodies) with lymphokine production and proliferation suggests that these cells are functionally mature.[13–16,18,19] In the murine system, the early appearance of the $\gamma\delta$ TCR in ontogeny suggests that these cells may be functionally important in fetal life;[20] however, a definitive physiologic role for $\gamma\delta$-bearing T cells has not been demonstrated in either the murine or human systems, nor has the normal physiologic distribution of these cells or their possible participation in conventional lymphoid responses been elucidated.

This report immunohistologically confirms the presence of TCR-δ^+ cells in both the human thymus and peripheral lymphoid organs. In the thymus, these cells were located primarily in the medulla and in the juxtamedullary cortex rather than the subcapsular zone, which is the location of the most immature thymocytes. In the periphery, small numbers of TCR-δ-bearing cells (less than 5% of $CD3^+$ cells overall) were distributed in a fashion similar to conventional TCR-β^+ T cells (ie, predominantly located in classical T zones). Together, these data support the hypothesis that TCR-δ^+ cells constitute a distinct subpopulation of maturing and mature T cells, rather than a precursor population to TCR-$\alpha\beta$ T cells.

Because the number of TCR-δ^+ cells identified in reactive lymphoid processes in the tissues was equal or less than the number reported in peripheral blood (2–4%),[4,5,8] these results are not consistent with selective recruitment of TCR-δ^+ cells in any of the cases examined nor did the numbers of TCR-δ^+ cells correlate with location in the body. Finally, given the suggestion that $\gamma\delta$ T cells may be important in immune surveillance of neoplasia,[6,14,15,18] the number of reactive TCR-δ^+ cells within 9 B lineage and 59 TCR-δ^- (see below) T lineage lymphomas were assessed. Although scattered TCR-δ^+ cells were almost always identified, the percentage relative to conventional T cells was always very small (data not shown), a finding arguing against preferential participation of TCR-$\gamma\delta^+$ in tumor-related immune responses.

A primary focus of this study was the analysis of TCR-δ expression in T lineage lymphomas. The authors reported previously that malignant T lineage proliferations frequently lacked TCR-β expression, or in some cases showed TCR-β expression in the absence of CD3.[3] Because cytoplasmic expression of both CD3 and TCR-β appears to occur quite early in the intrathymic development of conventional T cells (greater than 90–95% of thymocytes display, at least in the cytoplasm, both antigens),[2,3] the finding of CD3/TCR-β discordant neoplastic cells with an otherwise mature phenotype ($CD4^+/CD8^-$ or $CD4^-/CD8^+$) suggested the possibility of abnormal or at least asynchronous gene expression in these tumors.[3] This impression was supported by the finding that many of these lymphomas lacked other T cell antigens usually present in all or nearly all thymocytes and T cells (eg, CD2 or CD5).[21] It was possible, however, that the relatively high incidence of $CD3^+/TCR\text{-}\beta^-$ cases identified (30%) reflected preferential transformation of the physiologic TCR-δ-expressing subset. The results in this study argue against this hypothesis as only 2 of 14 $CD3^+/TCR\text{-}\beta^-$ lymphomas were demonstrated to be TCR-δ^+. It is unlikely that a significant subset of $\gamma\delta$-TCRs were missed with the TCR-δ-specific antibody, because as mentioned previously, this reagent reacts with all known TCR-$\gamma\delta$-bearing T cell clones or lines. Of the 12 $CD3^+/TCR\text{-}\beta^-$ cases that also lacked TCR-δ expression, six were phenotypically immature ($CD4^-/CD8^-$ or $CD4^+/CD8^+$) suggesting that these tumors may reflect transformation of that physiologic immature thymocyte subset caught between expression of CD3 and TCR-β. The six remaining $CD3^+/TCR\text{-}\beta^-$/TCR-δ^- cases showed a mature $CD4^+/CD8^-$ phenotype and five were morphologically mature (ie, nonlymphoblastic). These cases probably reflect either abnormal antigen expression or the presence of a unique, previously undescribed TCR not recognized by either the TCR-β or δ-specific antibodies. Prospec-

tive identification of these cases so that immunoprecipitation experiments with CD3 antibodies could be performed probably would resolve this issue.

It is of interest that the two TCR-δ^+ lymphoblastic lymphomas identified in this study, as well as the TCR-$\gamma\delta^+$ lymphoblastic leukemias reported by Gonzales-Sarmiento et al[22] were $CD4^-/CD8^-$, a phenotype associated with normal TCR-$\gamma\delta$ cells. Although this finding may in fact represent a physiologic association, only 2 of 11 $CD4^-/CD8^-$ neoplasms in this study were TCR-δ^+. Of the nine TCR-$\delta^-/CD4^-/CD8^-$ lymphomas, five were TCR-β^+ (3 PTL, 2 lymphoblastic) and four lacked both TCR-β and δ (2 PTL, 2 lymphoblastic). Thus, among T lineage lymphomas, the $CD4^-/CD8^-$ subset is heterogenous and may reflect the malignant transformation of early thymocytes, more mature TCR-$\gamma\delta$ expressing T cells, or other mature subsets of T cells whose phenotype has been altered by the induction of phenotypic aberrancy (ie, antigen loss) during transformation and expansion of the neoplastic clone.

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